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AUTHOR(S):

HIGASHI, Ohajime; TAKAGAKI, Teruyoshi; KOSHIMIZU, Koichi; WATANABE, Keisuke; KAJI, Mikio; HOSHINO, Jiro; NISHIDA, Toshisada; ... TAKASAKI, Hiroyuki; JATO, Johnson; MUANZA, Dave N.

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BIOLOGICAL ACTIVITIES OF PLANT EXTRACTS FROM TROPICAL AFRICA

Hajime OHIGASHI[i], Teruyoshi TAKAGAKI[i], Koichi KOSHIMIZU[i], Keisuke WATANABE[ii], Mikio KAJI[iii], Jiro HOSHINO[iv], Toshisada NISHIDA[v], Michael A. HUFFMAN[v], Hiroyuki TAKASAKI[vi], Johnson JATO[vii], & Dave N. MUANZA[viii]

ABSTRACT An investigation of plants in tropical Africa was conducted to search for new naturally bioactive substances. A total of 62 plant species were obtained from Cameroon, Zaire, and Tanzania. The extracts were tested for insecticidal, herbicidal, and fungicidal activities. Insecticidal activity was found in 23 extracts. Of the 51 extracts from Cameroon and Tanzania, herbicidal activity was detected in seven, and fungicidal activity in seven. *Polyascias fulva, Crassocephalum manii, Vernonia amygdalina, Vernonia vogetti, Poga oleosa, Gnidia glauca, Trema guinensis* and *Combretum bracteatum* were evaluated to be highly promising candidates for further investigation of their active constituents on the basis of their potency and/or broad spectrum of biological activities.

Key Words: Tropical African plants; Insecticidal activity; Herbicidal activity; Fungicidal activity; Medicinal plants.

INTRODUCTION

The tropical forest is one of the most interesting places in the world from the standpoint of bioactive natural product research. It is rich in plant species which are adaptive in a coevolutionary environment with regard not only to climatic factors but also to complex biological interactions. Tropical plants are surrounded by various competitors and consumers, and may be endowed with peculiar strategies for growth and reproduction, perhaps much more so than plants in other environments. A chemical defense system may be one possible strategy. It is well known that secondary plant metabolites (e.g. alkaloids, terpenoids, flavonoids, etc.) play significant roles in the biosphere as defensive substances that deter mammals and insects (Harborne, 1977). Phytoncides or allelopathic substances act as deterrents against microorganisms or other plant species (Harborne, 1977). The plants with medicinal properties are frequently used by local inhabitants (Lewis & Elvin-Lewis, 1977; Watt & Breyer-Brandwijk, 1962). Thus, the plants in tropical

i) Department of Food Science and Technology, Faculty of Agriculture, Kyoto University; ii) Agricultural Science Research Laboratory, Sumitomo Chemical Co. Ltd.; iii) University Forest in Chichibu, Faculty of Agriculture, The University of Tokyo; iv) College of Liberal Art, Himeji Dokkyo University; v) Laboratory of Human Evolution Studies, Faculty of Science, Kyoto University; vi) Center for African Area Studies, Kyoto University; vii) Institute of Medical Research and Studies of Medicinal Plants; viii) Laboratoire de Recherche sur les Plants Medicinales, Section Laboratoire ISTM-Kinshasa.

forests are valuable sources of naturally occurring bioactive substances. Although bioactive constituents of tropical plants have been extensively investigated (Hostettman & Marston, 1990; Watt & Breyer-Brandwijk, 1962), research has by no means been exhausted. Recent advances in biochemical and physiochemical methodology make it possible to investigate biologically and physiologically active plant constituents for new modes of activity.

Since 1983, we have conducted investigations to search for useful plants in the tropical rain forest of Cameroon (Ohigashi et al., 1987). Several new plant constituents with biological and physiological significance have been isolated from those plants (Koshimizu et al., 1992; Murakami et al., 1991a, 1991b; Ohigashi et al., 1989, 1991).

As a part of this long-term research, a third, more extensive field survey in Cameroon was carried out in 1988. This survey was conducted at two sites near Nkoélon and Nkanbé. Nkoélon, located in the rain forest of southwestern Cameroon, is close to Mvini village, a site at which previous surveys in 1983 and 1985 were conducted. Kaji indicated that the vegetation of this area is quite similar to that of the Mvini area (Kaji, 1990). At the Nkoélon area, random collection of plants (27 species) was undertaken, because the survey of useful plants in this area had already been completed (Ohigashi et al., 1987). On the other hand, the vegetation of the Nkambé area, located at the northwestern margin of the rain forest, is quite different from that at Nkoélon and can be characterized as either Afromontane or Afro-submontane (Letouzey, 1980). Medicinal plants in this area have been extensively surveyed by Jato (Jato, 1988). Furthermore, a preliminary survey in 1985 suggested that medicinal plants in the Nkambé area exhibited several biological activities at high rates (Ohigashi et al., 1987). Hence, further collection of medicinal plants (20 species) in this area was made.

Additional plant species were supplied from other African countries. One group of medicinal plants (11 species) were collected around Kinshasa, Zaire at the southern margin of the Central African rain forest by Muanza, and another group of plants (five species) containing four possible medicinal plants used by wild chimpanzees (Huffman & Sefu, 1989; Nishida, 1990; Takasaki & Hunt, 1987; Wrangham & Nishida, 1983) were collected from an area of savanna woodland in the Mahale Mountains National Park, Tanzania, by Nishida, Huffman and Takasaki.

This report describes insecticidal, herbicidal and fungicidal activities of these plant extracts, which were tested by our standard bioassay systems (Ohigashi et al., 1987).

MATERIALS AND METHODS

I. Preparation of Plant Extracts

All dried plant materials (usually 100 g dry weight) were immersed in methanol for 10–20 days at room temperature and then the extract solution was concentrated *in vacuo*. These methanol extracts were then submitted to the following bioassays,

and the activities in each assay were ranked by the standards as shown below.

II. Insecticidal Tests

Insecticidal activity was tested not only for the tobacco cutworm and the northern house mosquito, as conducted previously (Ohigashi et al., 1987), but also for the housefly and the pyrethroid-resistant diamondback moth.

1. Larval-Growth Inhibitory Activity against the Tobacco Cutworm (*Spodoptera litura*)

An emulsified aqueous solution (2 ml) of a test compound was mixed with a dose of artificial diet (13 g) and placed in a plastic cup (100 mm i.d., 35 mm depth) with ten of the 4th instar larvae. The test was usually conducted at a concentration of 4,000 ppm. In some cases, a concentration of 2,000 ppm was used because of limited sample availability. The mortality rate of the larvae and the feeding inhibitory rate to a control experiment were measured after six days. Inhibitory activity against larval growth measured by the mortality rate was evaluated using the following four ranks: +3, 100% mortality; +2, 90%–99% mortality; +1, 50–89% mortality; and − (inactive), less than 49% mortality. Feeding inhibition measured by the feeding speed was evaluated in four ranks: +3, more than 90% inhibition; +2, 50–89% inhibition; +1, 10–49% inhibition; and − (inactive), less than 9% inhibition.

2. Larval-Growth Inhibitory Activity against the Northern House Mosquito (*Culex pipiense pallense*)

Twenty 4th instar larvae were released in an emulsified aqueous solution (100 ml) of a test compound. The test was usually conducted at a concentration of 20 ppm. Some extracts, limited by availability, were tested at a reduced concentration (10 ppm) as in the case of the tobacco cutworm assay. After 24 hrs, the inhibitory activity of larval growth measured by the mortality rate was evaluated by the following four ranks: +3, 100% mortality; +2, 90–99% mortality; +1, 10–89% mortality; and − (inactive), less than 9% mortality. Thereafter, the surviving larvae were further reared until adult emergence. Inhibition of adult emergence compared to a control experiment was evaluated in four ranks: +3, more than 90% inhibition; +2, 80–89% inhibition; +1, 10–79% inhibition; and − (inactive), less than 9% inhibition.

3. Larval-Growth Inhibitory Activity against the Diamondback Moth (*Plutella xylostella*)

Leaf disks (2 cm in diameter) were punched out from a cabbage leaf. Both surfaces of the disks were treated with 20 μl of a 5,000 ppm solution of the methanol extract. Three treated disks and three untreated disks were alternately placed per petri-dish (10 cm in diameter) in which ten 3rd instar larvae were released. Inhibitory activity against larval growth measured by the mortality rate after two days was evaluated in four ranks: +3, 100% mortality; +2, 90–99% mortality; +1, 60–89% mortality; and − (inactive), less than 59% mortality. Feeding

inhibition was measured after five days by the percentage of leaf area consumed and evaluated in four ranks: +3, no feeding; +2, less than 10% consumed; +1, 11–80% consumed; and − (inactive), more than 81% consumed.

4. Killing Activity against the Adult Housefly (*Musca domestica*)

Thirty milligrams of the plant extracts in 1 ml of acetone was applied to bait consisting of 2 g of powder milk and 1 g of sugar placed on an aluminum tray. After air-drying, the bait and a cup of water (5 ml) were placed in a cage into which 50 housefly pupae were put. After two days, the adults emerged, and their mortality rate was determined after another seven days. Killing activity against the adult housefly was evaluated in four ranks: +3, more than 90% mortality; +2, 50–89% mortality; +1, 30–49% mortality; and − (inactive), less than 29% mortality.

III. Herbicidal Tests

Herbicidal activity was tested basically by the same methods previously reported (Ohigashi et al., 1987). A sanitary cotton sheet (50 mm × 50 mm) containing 0.1 ml of an emulsified solution (Tween 80-acetone, 1:10) of a test compound and 10 ml of water was placed into a glass tube (70 mm i. d., 100 mm depth). Normally the test was started at a concentration of 1,000 ppm. Seeds of test plants [three seeds of cucumber (*Cucumis sativus*), seven seeds of barnyard grass (*Echinochloa utilis*), 30 seeds of mustard (*Brassica junea*) or carrot (*Daucus carota* var. *sativa*)] were placed on the cotton. They were incubated at 27–28°C under a white fluorescent light (about 3,000 lux) for ten days. The rates of germination and the growth of the aerial part and/or the root were compared to control experiments. When growth inhibition was detected at 1,000 ppm, tests at further diluted concentrations were conducted. Activity was evaluated in four ranks: +3, more than 50% inhibition at 250 ppm; +2, more than 50% inhibition at 500 ppm; +1, more than 50% inhibition at 1,000 ppm; and − (inactive), less than 49% inhibition at 1,000 ppm.

IV. Fungicidal Tests

Fungicidal tests were conducted against ten species of phytopathogenic fungi by spraying emulsified extract solutions (500 ppm) on the host plants. The method used (Ohigashi et al., 1987) was basically the same in all of the tests, except for the host plant species and incubation conditions. Preventive effects on diseases by the fungi, *Pyricularia oryzae, Rhizoctonia solani, Cercospora arachidicola, Botrytis cineria, Phytophtora infestans* and *Venturia inaequalis*, were investigated. A typical run for *P. oryzae*: The emulsified aqueous solution of the extract (500 ppm) was sprayed onto the foliage of a host plant, *Oryza sativa*. After air-drying, spores of *P. oryzae* dispersed in water were sprayed onto the plant treated with the extract. The plant thus treated was incubated in a dark humidified room (99% humidity) for four days at 23°C. The preventive effect of the extract on the fungus disease was observed. Host plants and incubation conditions for the other fungi species were as follows: *C. arachidicola* on *Arachis hypogoea* for four days at 23°C

in a dark humidified room; *B. cineria* on *Cucumis sativus* for four days at 23°C in a dark humidified room; *P. infestans* on *Lycopersicum esculentum* for seven days at 23°C in a dark humidified room; *V. inaequalis* on *Malus domestica* for 18–20 days at 15°C in a greenhouse. In the case of *R. solani*, the spores precultured in a rice hull medium were inoculated on the base of the host plant, *Oryza sativa*, which was pretreated with an emulsified test solution of 500 ppm. The plant thus treated was incubated for seven days at 25–27°C in a humidified greenhouse (99% humidity).

The fungi, *Erysiphe graminis, Puccinia recondita, Pseudoperonospora cubensis* and *Plasmopara viticola* were used to identify the curative effects of extracts. The following is a typical test run: The spores of *E. graminis* were sprayed onto the foliage of the host plant, *Hordeum vulgare*. After preincubation in a humidified greenhouse (99% humidity) at 23–25°C for 18 hrs (preincubation), the emulsified aqueous solution of the extract (500 ppm) was sprayed onto a fungus infected plant. The plant thus treated was incubated at 23°C under continuous light (10,000 Lux) for ten days. The curative effect of the extract on the fungus disease was investigated. The host plants and incubation conditions for the other fungus were as follows: *P. recondita* on *Triticum aestivum*, preincubated for 18 hrs at 23–25°C in a humidified (99%) greenhouse and incubated for 12 days at 23°C under continuous light (10,000 lux); likewise, *P. cubensis* on *Cucumis sativus*, for 18 hrs at 23–25°C in a humidified (99%) greenhouse and additional three days at 23°C in the dark, followed by a further ten days at 28°C in a greenhouse; *P. viticola* on *V. vinifera*, for 18 hrs at 23–25°C in a humidified (99%) greenhouse and additional three days at 23°C in the dark, followed by further ten days at 28°C in a greenhouse.

The fungicidal activity was evaluated in six ranks: +5, 100% inhibition of the fungus disease; +4, 90–99% inhibition of the disease; +3, 70–90% inhibition of the disease; +2, 50–69% inhibition of the disease; +1, 20–49% inhibition of the disease; and − (inactive), less than 20% inhibition of the disease.

RESULTS AND DISCUSSION

All the plants from Zaire and Tanzania were identified. On the other hand, species identification of plants from Cameroon has been completed for 25 plants, and the genus was identified for eight plants. Further efforts to identify the plants are currently under way.

Table 1 shows the results of the standard bioassays for insecticidal, herbicidal and fungicidal activities of 48 plants, whose genera are identified. Unidentified plants were not listed except *APA, BOKKABOKUL* and *UNBAKFOLL* (vernacular name used at Nkoélon), which showed significant biological activities.

Insecticidal activities against four species of test insects were examined for all of the plant extracts (62 species). Against the tobacco cutworm, one of the standard test insects for insecticidal activity, 11 extracts were found to slightly inhibit larval growth, and 13 extracts inhibited larval feeding. Such effect on *Vernonia vogelli* was the most striking. Seven extracts exhibited inhibitory activity against larval

Table 1. Biological activities of the plant extracts.[a)]

Plant		Biological Activity								
		Insect[d)]								
		SL		CP		MD	PX			
Family Species (Part)[b)]	Place[c)]	M	D	M	E	M	D	Herb[e)]	Fung[f)]	Remarks[g)]
Anacardiaceae										
Mangifera indica (B)	Zai	−	−	−	−	−	−	NT	NT	Med
Annonaceae										
Annona senegalensis (B)	Zai	−	−	−	−	−	−	NT	NT	Med
Apocynaceae										
Rauwolfia vomitoria (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Araliaceae										
Polyascias fulva (B)	NW-Cam	−*	+1*	−*	+2*	+3	−	−	+3PC	Med
Combretaceae										
Combretum racemosum (B)	Zai	+1	+2	−	−	−	−	NT	NT	Med
Combretum bracteatum (L)	SW-Cam	−	−	−	−	−	−	+3BJ, +3EU +3CS	−	
Terminalia sp. (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Compositae										
Aegeratum sp. (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Aspilia mossambicensis (L)	Tan	−	−	+1	+2	−	−	+1BJ	−	Med-PM
Crassocephalum manii (B)	NW-Cam	−	−	−	−	−	−	−	+5PC	Med
Vernonia amygdalina (L)	Tan	+1	+2	+1	−	−	−	−	+4PC, +2RS	Med, Med-PM
Vernonia guineensis (L)	NW-Cam	−	−	−	−	−	−	−	−	Med
Vernonia vogelli (L)	SW-Cam	+1*	+3*	−*	−*	−	−	−	−	
Ebenaceae										
Diospyros sp. (L)	SW-Cam	−*	+2*	−*	−*	−	−	−	−	
Euphorbiaceae										
Alchornia cordifolia (L)	Zai	−	+1	−	−	−	−	NT	NT	Med
Bridelia ferruginea (B)	Zai	−	−	−	−	−	−	NT	NT	Med
Euphorbia hirta (B)	Zai	−	−	−	−	−	−	NT	NT	Med
Hymenocardia acida (B)	Zai	−	−	−	+1	−	−	NT	NT	Med
Manniophyton fulvum (B)	Zai	−	−	−	−	−	−	NT	NT	Med
Flacourtiaceae										
Casearia sp. (L)	SW-Cam	+1*	+2*	−*	−*	−	−	−	−	
Irvingiaceae										
Desbordesia sp. (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Labiatae										
Ocimum gratissimum (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Ocimum sp. (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Leguminosae										
Afzelia bipindensis (L)	SW-Cam	−*	−*	−*	−*	−	−	+1BJ, +1EU +1CS, +1DC	−	
Crotalaria coolata (B)	NW-Cam	−	−	−	−	−	−	+1BJ	−	
Cynometra sp. (L)	SW-Cam	−*	+1*	−*	−*	−	−	−	−	
Daniella olivery (L)	NW-Cam	−*	−*	−*	−*	−	−	−	−	Med
Dialium pachyphyllum (L)	SW-Cam	−*	−*	−*	−*	−	−	−	+3RS	
Distemonanthus benthamianus (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Liliaceae										
Allium sativum (L)	NW-Cam	−	−	−	−	−	−	−	−	Med

(**Table 1.** cont.)

Malvaceae										
Sida rhombifolia (L)	Zai	+1	−	−	−	−	−	NT	NT	Med
Moraceae										
Ficus exasperata (L)	Tan	−	−	+1	−	−	−	−	−	Med-PM
Myrtaceae										
Psidium guajava (L)	Zai	−	−	−	−	−	−	NT	NT	Med
Olacaceae										
Olax subscorpioidea (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Strombosiopsis tetrandra (L)	SW-Cam	−*	−*	−*	−*	−	−	−	−	
Rhizophoraceae										
Poga oleosa (L)	SW-Cam	+1*	+1*	+1*	+2*	−	−	−	−	
Rosaceae										
Pygeum africanum (B)	NW-Cam	−	−	−	−	−	−	+2DC	+3PC	Med
Rubiaceae										
Mussaendra arcuata (L)	Tan	−	−	+1	−	−	−	−	−	Med
Nauclea latifolia (L)	NW-Cam	+1*	+1*	+1*	−*	−	−	−	−	Med
Nauclea sp. (L)	NW-Cam	+1*	−*	−*	−*	−	−	−	−	Med
Rothmannia withfieldii (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Tricalysia grossweileri (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Sapotaceae										
Zanha africana (B)	NW-Cam	−	−	−	−	−	−	−	−	Med
Thymeraceae										
Gnidia glauca (L)	NW-Cam	+1*	+2*	+1*	−*	−	+1	−	+3VI	
Verbenaceae										
Lippia plicata (L)	Tan	−	−	−	−	−	−	−	−	Med-PM
Vitex madiensis (RB)	Zai	−	+1	−	−	−	−	NT	NT	Med
Ulmaceae										
Trema guinensis (B)	NW-Cam	+1*	+2*	+1*	−*	−	−	−	−	Med
Trema orientalis (B)	NW-Cam	−	−	+2	−	−	−	−	−	Med
APA (L)	SW-Cam	−*	−*	−*	−*	+1	+2	+2Cu +2BJ	+2RS	
BOKKABOKUL (L)	SW-Cam	−*	−*	−*	+1*	−	+1	−	−	
UNBAFOLL (L)	SW-Cam	+1*	−*	−*	−*	−	−	+2BJ +2EU	−	

a) Each activity is expressed by the signature indicated in the Materials and Methods. NT: not tested.
b) Part extracted. B; bark; L: leaf; RB: root-bark.
c) Place collected. Zai: Zaire; NW-Cam: northwest Cameroon (Nkambé); SW-Cam: southwest Cameroon (Nkoélon); Tan: Tanzania.
d) Insect: insecticidal activity. SL: *Spodoptera litura*; CP: *Culex pipiense pallense*; MD: *Musca domestica*; PX: *Plutella xylostella*; M: mortality; D: feeding-deterrent activity; E: emergence inhibitory activity; *: test at 2000 ppm for SL and 10 ppm for CP.
e) Herb: herbicidal activity. BJ: *Brassica junea*; CS: *Cucumis sativus*; EU: *Echinochloa utilis*; DC: *Daucus carota*.
f) Fung: fungicidal activity. PC: *Pseudoperonospora cubensis*; RS: *Rhizoctonia solani*; VI: *Venturia inaequalis*; PV: *Plasmopara viticola*.
g) Med: local medicinal plant; Med-PM: possible medicinal plant used by wild chimpanzees.

growth of the mosquito, and six against adult emergence. Two extracts possessed killing activity of the adult housefly. The activity of *Polyascias fulva*, a medicinal plant found at the margin of the rain forest of Cameroon, was quite high. Deterrent activity against larval feeding of the diamondback moth, a pest to several cultivated vegetables, was found in three extracts. Thus, 23 out of the 62 extracts showed some kind of insecticidal activity and the activity-exhibiting rate (AER; 37%) was much higher than that (11%) in previous testing (Ohigashi et al., 1987). This may largely be due to the fact that the number of plants collected at the margin of the rain forests and savanna woodland was larger than that in the previous survey (Ohigashi et al., 1987). Then, it may be suggested that insect populations, particularly pest, are densest in such areas where human activities are high, hence the plants in these areas are endowed with defense systems against such insect attacks. Actually, 15 of the 23 extracts found to be active in the insecticidal tests were those from the plants collected at the margin of the forest or in the savanna woodland. Moreover, it may be proposed that some insecticidally active substances also possess significant value of physiological activity to humans. Among above 15 species, 11 species were locally used as medicinal plants (Jato, 1988) (Table 1).

Herbicidal activities, tested in cucumber, barnyard grass and carrot seedlings were found in seven out of 51 plant extracts. The AER was 13.7%. This rate was quite low compared with that (38%) of the previous study (Ohigashi et al., 1987). In this case, the fact that a fewer number of plants were collected from the rain forest may be again pointed out. These results may reflect that competition between plants is much more severe in the forest than the margin or outside of the forest, and plant growth inhibitory factors in the forest are much abundant. Among the herbicidally active plants, *Combretum bracteatum*, collected at Nkoélon, was most remarkable because of both the potency and the chemical characteristic of water solubility of the active constituent(s).

Fungicidal tests using 51 extracts against ten phytopathogens showed significant activity in seven extracts. Similarly to herbicidal activities, the AER (17.6%) in fungicidal tests was lower than that (31%) of the previous study (Ohigashi et al., 1987), suggesting severe competition between plant and microorganism in the rain forest. Of the 7 active extracts, those of *Crassocephalum manii* and *V. amygdalina* were indicated to contain invaluable active substances.

As a result of the biological activity tests, a total of 31 out of 62 species exhibited some kind of biological activity in the assays performed. The active constituents of *P. fulva, C. manii, V. amygdalina, V. vogetti, P. oleosa, Gnidia glauca, Trema guinensis* and *Combretum bracteatum* were evaluated to be important candidates for further study on the basis of the potency of each activity and/or the broad spectrum of activities displayed. It is hoped that these results will provide significant information for many areas of research in tropical Africa.

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Authors' Names and Addresses: Hajime OHIGASHI, Teruyoshi TAKAGAKI, Koichi KOSHIMIZU*, *Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606–01, Japan*; Keisuke WATANABE, *Agricultural Science Research Laboratory, Sumitomo Chemical Co. Ltd., Takarazuka Hyogo 665, Japan*; Mikio KAJI, *University Forest in Chichibu, Faculty of Agriculture, The University of Tokyo, Chichibu, Saitama 368, Japan*; Jiro HOSHINO, *College of Liberal Art, Himeji Dokkyo University, Himeji, Hyogo 670, Japan*; Toshisada NISHIDA, Michael A. HUFFMAN, *Laboratory of Human Evolution Studies, Faculty of Science, Kyoto University, Kyoto 606–01, Japan*; Hiroyuki TAKASAKI, *Center for African Area Studies, Kyoto University, Kyoto 606–01, Japan*; Johnson JATO, *Institute of Medical Research and Studies of Medicinal Plants, BP 292, Yaounde, Republic of Cameroon*; Dave N. MUANZA, *Laboratoire de Recherche sur les Plants Medicinales, Section Laboratoire ISTM-Kinshasa, P. O. Box 774, KIN XI, Kinshasa, Zaire*.

* To whom all correspondence should be addressed.